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An electron microscopic study of microbody types found in dwarf pea internode.

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AN ELECTRON MICROSCOPIC STUDY OF MICROBODY TYPES FOUND IN DWARF PEA INTERNODE

A Thesis

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and the

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In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

by

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THESIS ACCEPTANCE

Accepted for the faculty of The Graduate College of the University of Nebraska at Omaha in partial fulfillment of the requirements for the degree Master of Arts

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4 December 1975
Date

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LIST OF ABBREVIATIONS

Ch	chloroplast
CCB	crystal containing body
CW	cell wall
D	dictyosome
ER	endoplasmic reticulum
gr	chloroplast grana
M	mitochondria
Mb	microbody
N	nucleus
P	plastid

INTRODUCTION

Ever since plant cell organelles morphologically resembling animal microbodies were clearly established by Mollenhauer et al. (1966) and Frederick et al. (1968), a great deal of attention has been focused on this subject. Several papers outlining morphology and orientation have followed (Frederick and Newcomb, 1968, 1969; Vigil, 1971, 1973a). Plant microbodies appear to be a distinct class of organelle found ubiquitously in cells. They are single membrane-bounded and have a dimensional range of 0.2u - 0.5u. Their interior exhibits a coarse to finely granular matrix and often a para-crystalline or nucleoid structure is quite evident. Microbodies are characteristically associated with smooth or rough endoplasmic reticulum and are often found in close proximity to mitochondria and/or chloroplasts. Frederick (1968) has shown some evidence of a possible endoplasmic reticulum origin for microbodies.

Extensive biochemical studies upon isolated organelles, obtained through ultra-centrifugation in a sucrose gradient, have been carried out by Breidenbach and Beevers (1967) and Tolbert et al. (1968). As a result, microbodies were divided into a minimum of two classes, based upon their enzyme compliment. Those organelles containing enzymes related to the Kornberg and Krebs glyoxylate cycle were termed "glyoxosomes." Glyoxosomes have characteristically been associated with developing embryonic tissue and tissue utilizing acetate as a carbon source. In embryo tissue, they function in the oxidation of reserve lipid to succinate and malate and thus utilize the glyoxylate pathway (Vigil, 1973b).

The other biochemically distinctive class of microbodies contains large quantities of catalase and peroxidase. For this reason, such microbodies have been called "peroxisomes" (De Duve and Baudhuin, 1966). Tolbert and Yamazaki (1969) indicated that peroxisomes were responsible for the aerobic oxidation of glycolate excreted from the chloroplast as a result of photorespiration under low carbon-dioxide concentrations.

Based strictly upon a morphological appearance, there is yet a third group of microbodies, the crystal-containing bodies. These organelles are morphologically like other microbodies but for the exception that they contain a para-crystalline structure. These microbodies, referred to as CCB (Thornton and Thimann, 1964), are peroxisomal in nature as evidenced by histochemical staining with 3,3' diaminobenzidine tetrahydrochloride, DAB (Vigil, 1969). DAB has a specificity for peroxidatic activity. The crystal-line structure within these microbodies shows a high concentration of such activity. Therefore, this reaction of the crystalloid with DAB suggests that these para-crystalline structures may be crystallized catalase or peroxidase (Tolbert, 1971; Vigil, 1973b).

This electron microscopic survey of microbody development is proposed in order to give a clearer picture of what occurs in herbaceous stem from embryonic tissue through early senescence. It is also interesting to note that at present, no evidence of CCB presence in the legume family has been documented.

METHODS AND MATERIALS

Seeds of dwarf pea (Pissum sativum L. var. Little Marvel) were obtained from Henry Field Seed and Nursery Company, Shenandoah, Iowa. The seeds were soaked for 24 hours in Tween-20 and placed in enriched soil. Germination and subsequent growth was carried out under greenhouse conditions. Specimens were harvested at three day intervals at 5 p.m. beginning with day 6 and continuing through day 24.

Immediately following their internode length measurement, the stem was sectioned into 1-2 cubic millimeter blocks and placed in 3% glutaraldehyde. Post-fixation rinsing and post-osmium tetroxide treatment rinsing was carried out in 0.025 M phosphate buffer at a pH of 6.8. Fixation in glutaraldehyde for a period of 24 hours was followed by washing with four changes of buffer, one hour duration each.

Post-fixation was done in 2% osmium tetroxide for four hours, followed by dehydration in an alcohol series

(Mollenhauer, 1964). Then a final dehydration in graded Epon 812 before embedding in same (Nordahl, 1969).

Sections were cut to 900-600 A as judged by interference colors of pale gold to silver-gray. Sectioning was accomplished on a Sorvall MT-2B Porter-Blum Ultramicrotome, using a glass knife. Sections were mounted on uncoated 400 mesh copper grids and stained in 2% uranyl acetate for 20 minutes. This was followed by 10 minutes in Reynold's lead citrate. The grids were then examined in a Hitachi HS-7S electron microscope at 50 KV with a 20u or 30u aperature. Resulting micrographs were developed in D-19 developer.

Observations were confined primarily to parenchymatous cells in the cortical region of the third internode.

RESULTS

From the examination of the growth curve in Figure 1, it appears that the third internode is approaching maturity between day 15 and day 21. As evidenced by the decreasing amount of elongation per unit time in Table I, the tissue beyond this point can be considered mature and has begun a metabolic decline

To further illustrate this, Figure 2A depicts a micrograph of 6 day old tissue; notice the immature, developing chloroplasts, the thin homogeneous cell wall, and the relatively large mitochondria. Now compare the 6 day micrograph with Figure 2B. Figure 2B is a micrograph of 15 day old tissue; notice the large chloroplasts with well developed grana, the compressed cytoplasm resulting from a well developed tonoplast, a cell wall evidencing a dark mid-area of secondary wall formation, and the relatively small mitochondria. The line in the lower left cover of the micrograph represents one micron.

The early, immature parenchyma showed an abundance of microbodies as evidenced by the micrographs shown in Figures 2A, 3A, 3B and 3C. However, there were no microbodies observed in the tissue examined from day 9 (Figure 3D), but it must be assumed that they are present as later observations showed microbodies present in day 12 (Figure 4A and 4B).

Figure 3C, a micrograph taken from 6 day tissue, portrays a microbody containing a nucleoid structure. These structures, while present in the 6 day tissue, were not abundant and they were not observed in the more mature tissue.

As the tissue progressed in age, the frequency of microbodies being observed decreased. And by day 18, Figure 4D, no hard evidence of their presence could be established.

It also should be mentioned that as the tissue matures, especially beyond day 12, depicted by Figure 4A and 4B, it becomes increasingly more difficult to work with because of the extreme vacuolation of these cells at this point of maturity. This vacuolation contributes to poor cell preservation.

The day 15 specimens, Figure 4C, showed the last observed presence of microbodies.

Figure 4D, an example of 18 day specimen, shows two well developed chloroplasts with starch crystal inclusions. The bodies adjacent to the chloroplasts are unidentifiable.

The last micrograph shown, Figure 5, is from 21 day old tissue. Note the thickness of the cell wall, not entirely due to an oblique cut, and the compression of the cytoplasm.

Figure 1. Time-Growth course of elongation in the third internode.

Table I. Observations on total length of the third internode Standard deviations of true mean are given after each datum.

Figure 1

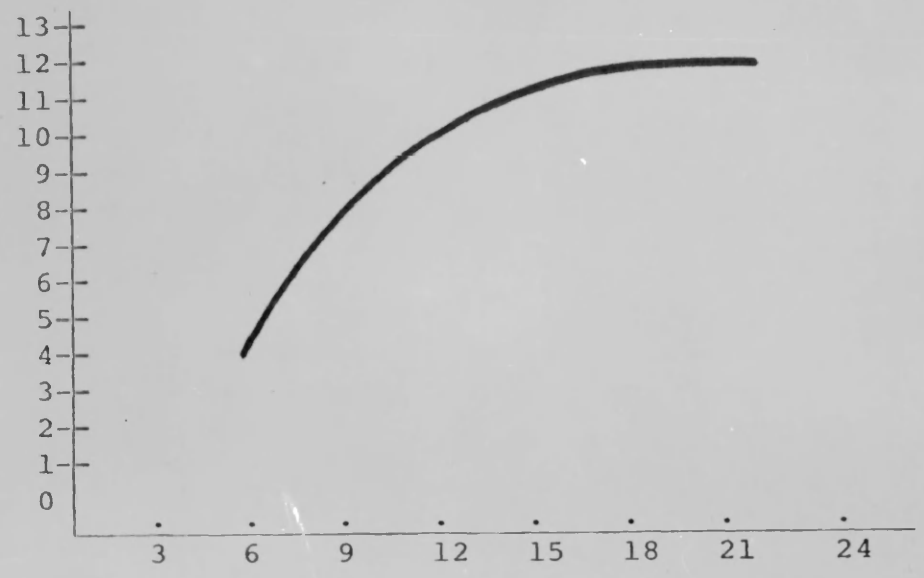


Table I

DAY	LENGTH (mm)
6	4.2 ± 0.3
9	7.5 ± 0.2
12	10.7 ± 0.3
15	10.7 ± 0.3
18	11.5 ± 0.3
21	12.7 ± 0.6
24	11.5 ± 0.6

Figure 2A. A cross section of parenchymous cell from 6 day old tissue. Line represents one micron.

Figure 2B. A cross section of parenchymous cell from 15 day old tissue. Note well developed grana. Line represents one micron.

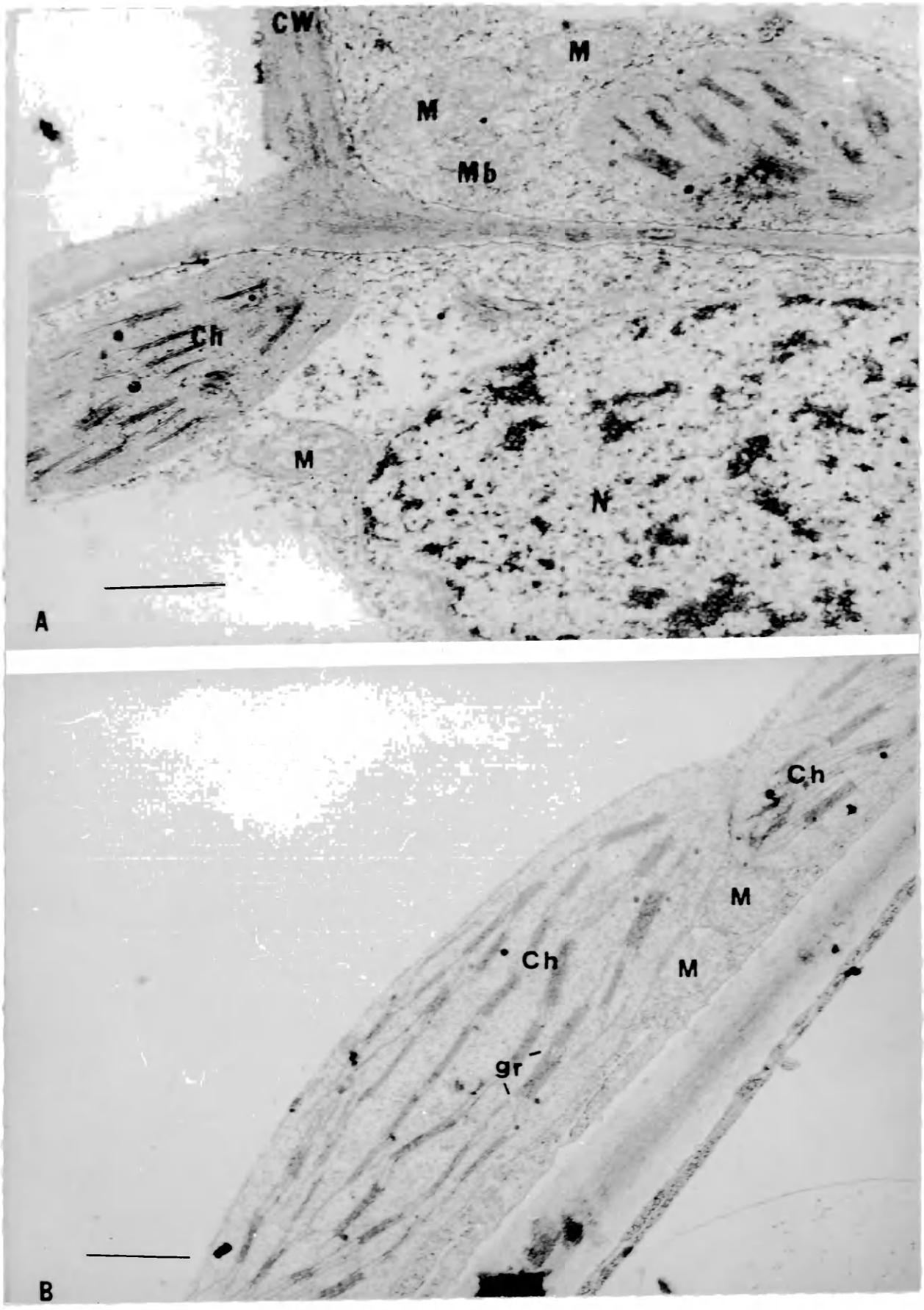

CW
M
M
Mb
Ch
M
N
A
Ch
M
Ch
M
gr
B

Figure 3A. Taken from a cross section of 6 day tissue; note the prominent microbody and the developing plastid.

Figure 3B. Cross section of 6 day cell. Note the thin cell wall and relative size of prominent microbody to mitochondria.

Figure 3C. Note microbody containing nucleoid structure in this cross section of a 6 day cell. Also note the relative position and size of the microbody to the adjacent mitochondria. Line represents one-half a micron.

Figure 3D. Two chloroplasts from a 9 day parenchyma.

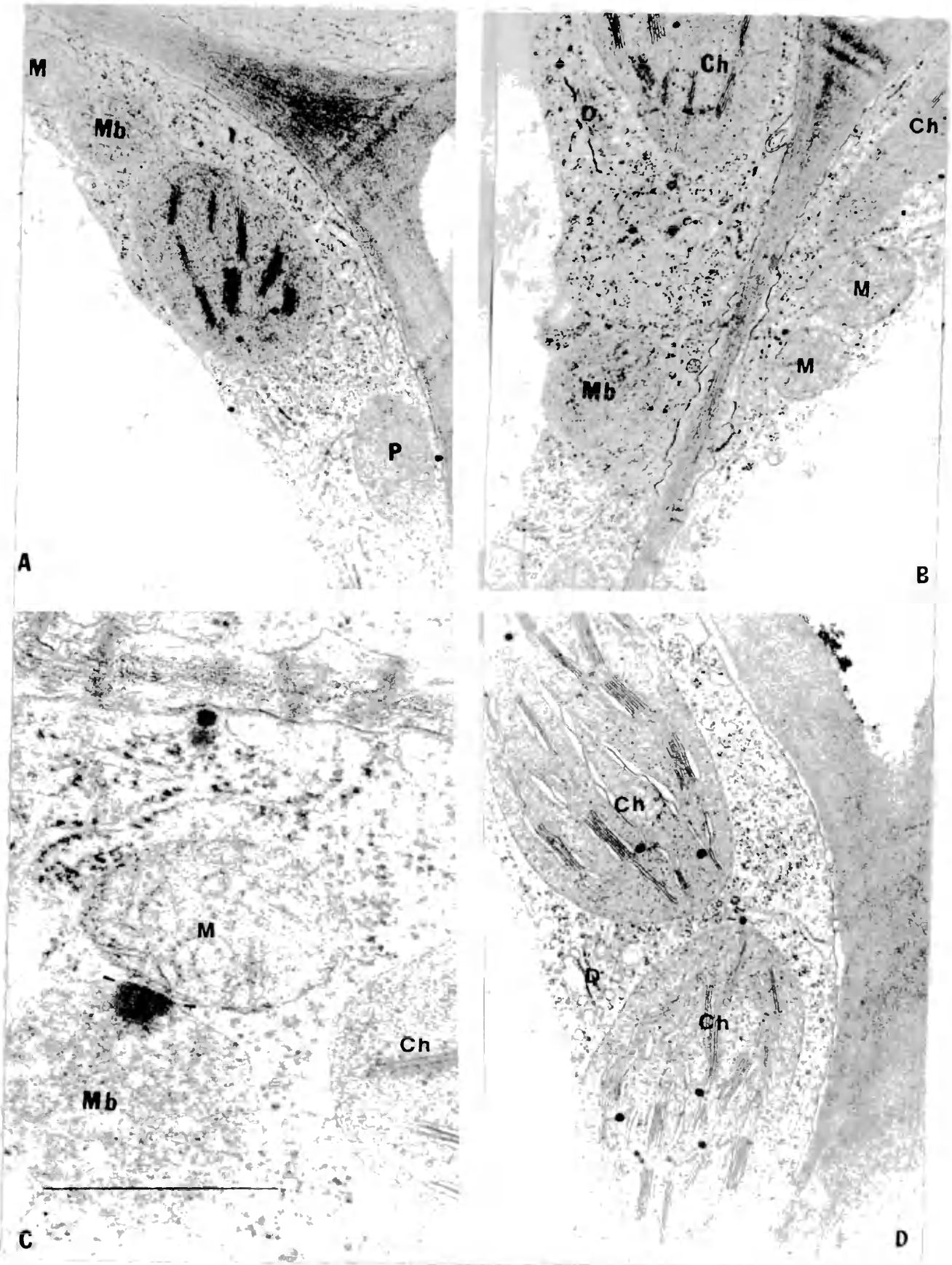

M
Mb
P
A
Ch
Ch
M
M
Mb
B
M
Ch
Mb
C
Ch
Ch
D

Figure 4A. Taken from 12 day tissue, this micrograph depicts a well defined microbody. Note the relative position to chloroplast. Line represents one micron.

Figure 4B. An example of 12 day parenchyma. Possible microbody is not well defined.

Figure 4C. Micrograph of 15 day old cell, last observed microbody. Note deterioration of micrograph quality due to poor tissue preservation.

Figure 4D. An example of 18 day old cell. Two well developed chloraplasts exhibiting starch granule inclusion. Bodies adjacent to chloroplasts are unidentifiable.

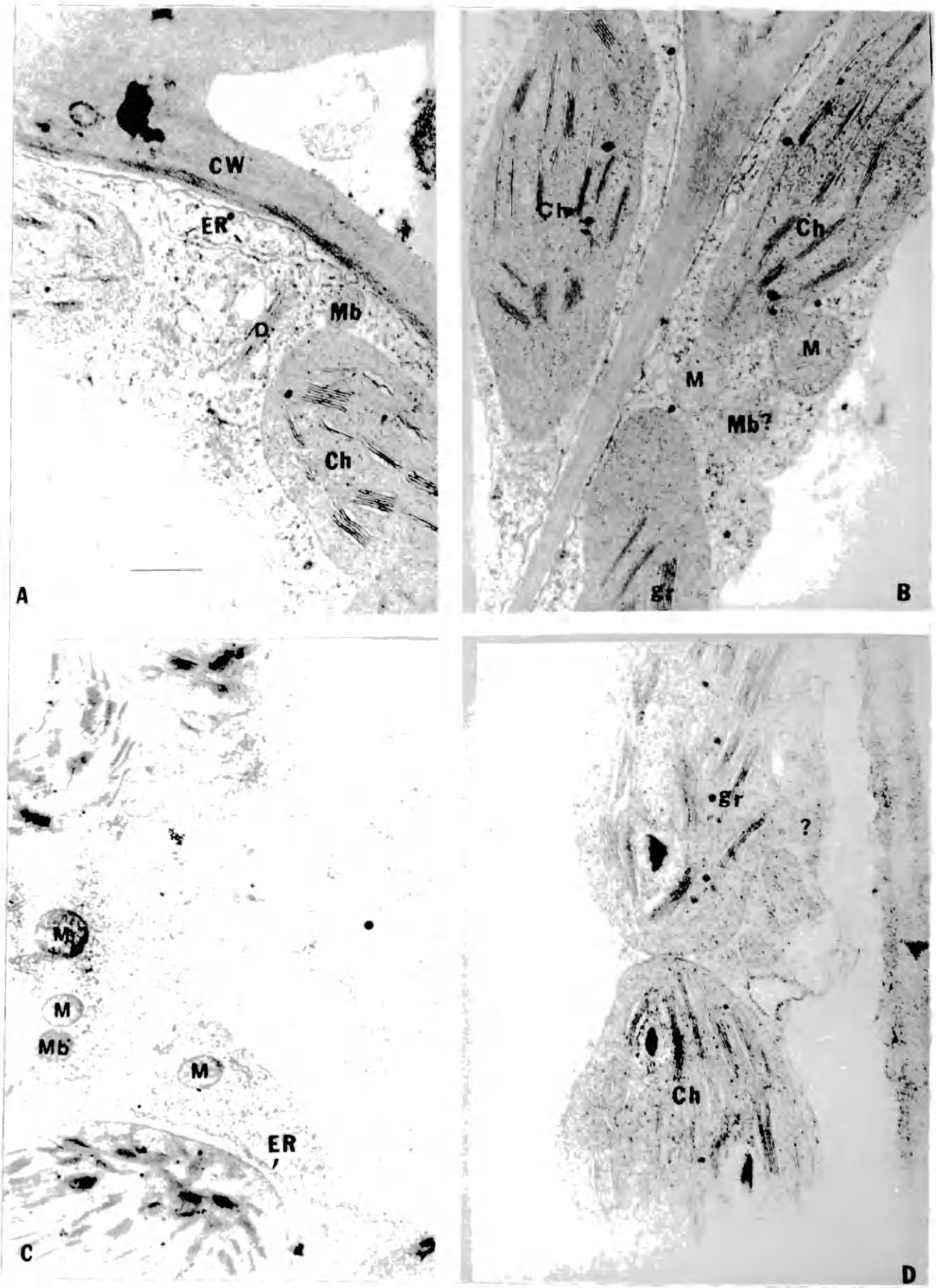
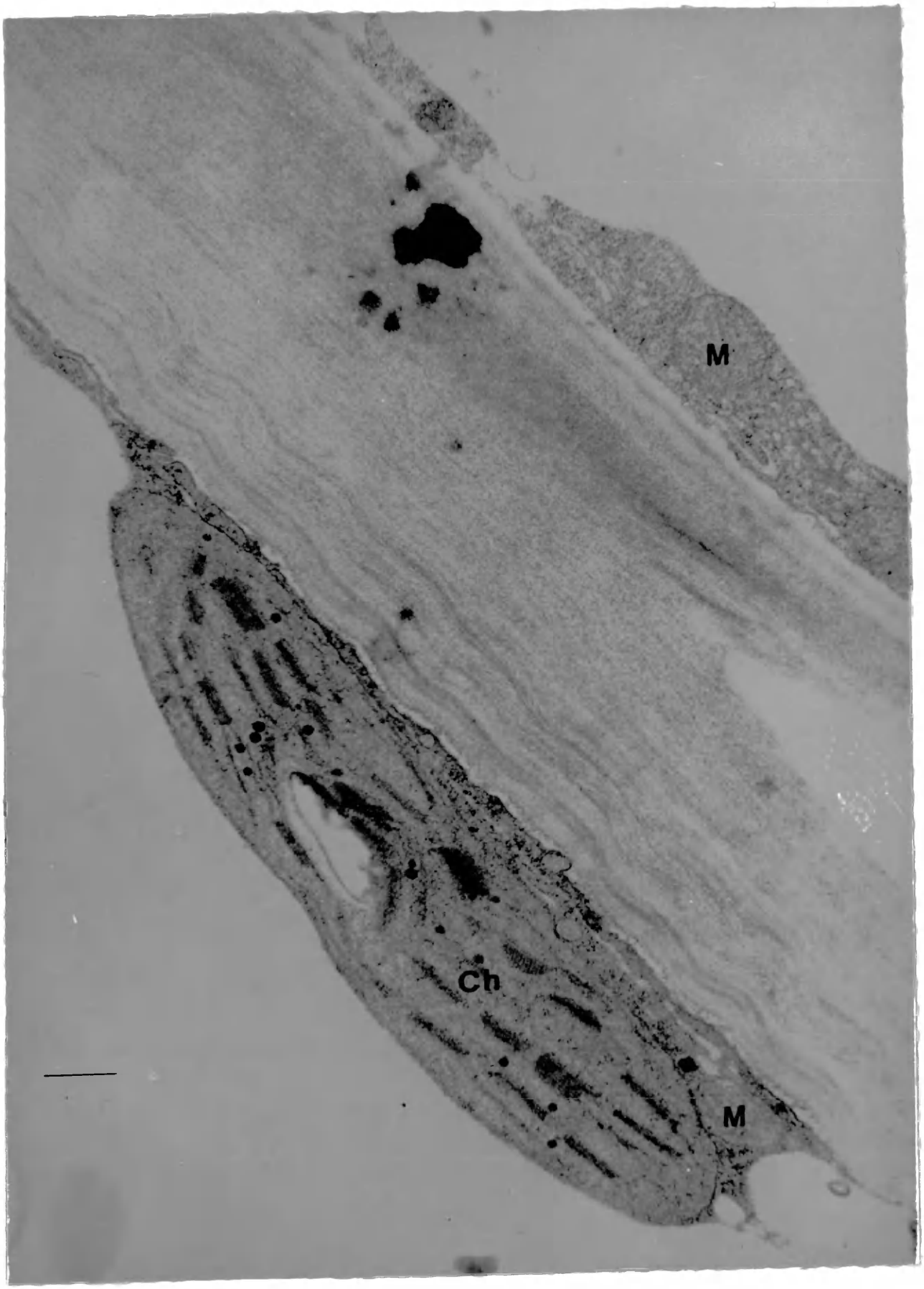

CW
ER
Mb
D
Ch
A
Ch
Ch
M
M
Mb?
gr
B
Mb
M
Mb
M
ER
C
gr
?
Ch
D

Figure 5. Large chloroplast in a thick section. Note the contour and thickness of the cell wall. Line represents one micron.


M
Ch
M

CONCLUSIONS AND DISCUSSION

The examinations leading to the preceeding micrographs have shown that as the parenchymatous cells age, the number of microbodies present decrease. This conclusion supports the work done by Breidenbach and Beevers (1969) in which isocitrate lyase, a glyoxosomal enzyme, activity was shown to decrease in castor bean after five days growth.

We can also assume the microbodies observed in the early stages of plant growth to be glyoxosomes, because glyoxosomes are primarily found in embryonic tissue and large numbers are seen in seedlings of plants whose energy source is lipid in nature (Trelease et al. 1971). The legumes are of this group.

Therefore, the resultant microbody decline that comes with age and maturity could be coupled with the decrease in metabolic demands of growth and elongation. Once the cell tissue no longer must supplement its energy

requirement with a lipid energy source, or the lipid source is depleted, the need for the glyoxolate pathway is lost. This results in a decrease of microbody numbers.

Also of interest, is the fact that no crystal containing bodies were observed. It has already been stated that CCB are peroxisomes (Vigil, 1969). Because of its crystalline structure, CCB are easily seen and differentiated as microbodies. Therefore, since no CCB were encountered, it is possible to speculate that none were present and perhaps peroxisomes are also absent in the stem of the dwarf pea.

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